EXPERT REVIEWS

The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases

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Chemokines are small proteins that control several tissue functions, including cell recruitment and activation under homeostatic and inflammatory conditions. CXCL8 (interleukin-8) is a member of the chemokine family that acts on CXCR1 and CXCR2 receptors. CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, and CXCL7 are also ELR+ chemokine members that bind to these receptors, especially CXCR2. The majority of studies on the biology of CXCL8 and their receptors have been performed in polymorphonuclear leukocytes. However, many other cells express CXCR1/CXCR2, including epithelial, endothelial, fibroblasts and neurons, contributing to the biological effects of CXCL8. There is substantial amount of experimental data suggesting that CXCL8 and receptors contribute to elimination of pathogens, but may also contribute significantly to disease-associated processes, including tissue injury, fibrosis, angiogenesis and tumorigenesis. Here, we discuss the biology of CXCL8 family and the potential therapeutic use of antagonists or blockers of these molecules in the context of organ-specific diseases.

Keywords: acute and chronic inflammation • chemoattractants • clinical trials • CXCL8 • CXCR1 • CXCR2 • infection

CXCL8/IL-8 family & receptors: from discovery to molecular aspects

Chemokines are small molecules (8–12 kDa), first described as chemoattractant cytokines [1–3], which play an important physiological role in the biology of leukocytes and other cell types by controlling tissue homeostasis, cell recruitment and activation and guiding leukocyte movements under basal and inflammatory states [1,2,4]. The chemokine system in mammals is complex, comprising about 50 ligands, constitutively expressed or induced by different stimuli and 20 receptors with 7 trans-membrane domains, belonging to class A of the rhodopsin-like G protein-coupled receptors (GPCRs) [2,3,5]. Chemokine receptors are distributed on the cell surface and act as sensors of the external environment, acting dynamically to transmit extracellular chemical signals by activation of intracellular pathways, changing cellular program and phenotype [2,3].

CXCL8 was purified 10 years after the discovery of the first chemokine, CXCL4/PF4, and caused renewed interest in the chemokine field at the time [4]. CXCL8 was described by independent groups by different names: neutrophil-activating factor [6]; monocyte-derived neutrophil-activating peptide [7]; and monocyte-derived neutrophil chemotactic factor [8]. Experimentally, CXCL8 was induced in lipopolysaccharide (LPS)-stimulated monocytes and shown to induce neutrophil migration. Hence, it was considered to be neutrophil-specific [2–4]. It was subsequently shown to be produced by a wide range of cell populations and have multiple cell targets in addition to neutrophils [9]. This is a relevant point as many therapeutic strategies based on the CXCL8 target only neutrophilic diseases, but the potential for such therapies extend well beyond neutrophils, as it will be discussed below.

CXCR1 and CXCR2 were the first members of the chemokine receptor family to be cloned, sharing a high degree of homology with formyl peptide receptors. The human genes for CXCR1 and CXCR2 (*il8ra* and *il8rb*, respectively) have been mapped to chromosome 2 [10]. There is also one pseudogene inside the CXCR1/CXCR2 gene cluster, named *il8rp*, but it presents multiple points of

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Table 1. Organization of agonists and chemokine receptors from IL-8 family.

Systematic name	Alternate human names	Human receptors
CXCL1	GRO-α, MGSA, GRO1, NAP-3	CXCR2, ACKR1/DARC
CXCL2	GRO-β, MIP-2 α, GRO2	CXCR2, ACKR1/DARC
CXCL3	GRO-γ, MIP-2 β, GRO3	CXCR2, ACKR1/DARC
CXCL5	ENA-78	CXCR2, ACKR1/DARC
CXCL6	GCP-2	CXCR1, CXCR2, ACKR1/DARC
CXCL7	NAP-2, CTAPIII, β-TG	CXCR1, CXCR2
CXCL8	IL-8, NAP-1, MDNCF, GCP-1	CXCR1, CXCR2, ACKR1/DARC
Systematic name	**Alternate mouse names**	**Mouse receptors**
Cxcl1	KC	CXCR2, ACKR1/DARC
Cxcl2	MIP-2	CXCR2, ACKR1/DARC
Cxcl3	DCIP-1	CXCR2, ACKR1/DARC
Cxcl5	LIX	CXCR1, CXCR2, ACKR1/DARC
CXCL7	NAP-2, CTAPIII, β-TG	CXCR1, CXCR2
Endogenous noncognate ligands	**Names**	**Receptors**
PGP	PGP	CXCR1, CXCR2
MIF	Macrophage MIF	CXCR2

MIF: Migration inhibitory factor; PGP: Proline–glycine–praline.

mutations and stop codons, compromising protein synthesis and structure [11,12]. The close clustering of these genes and their high degree of homology suggests that they may have arisen by duplication of a common ancestral gene [10–12]. CXCR1 and CXCR2 are distinguished by their different selectivity for their chemokine ligands, with CXCR1 displaying a relative selectivity for CXCL8 (Table 1). Human CXCR1 binds two chemokines, CXCL6/granulocyte chemoattractant protein-2 (GCP-2) and CXCL8, with high affinity [13]. Human CXCR2 binds to CXCL6/GCP-2 and CXCL8 and to other CXCL1/growth-related oncogene-α (GRO-α), CXCL2/GRO-β, CXCL3/GRO-γ, CXCL5/epithelial cell-derived neutrophil-activating peptide-78 (ENA-78) and CXCL7/neutrophil-activating peptide-2 (NAP-2) [13–16]. The chemokines that bind to the CXCL8 receptors, CXCR1 and CXCR2, are commonly named CXCL8 family chemokines and they share a common ELR+ (glutamic acid–leucine–arginine) motif in their structure [2,3]. Recently, two endogenous noncognate ligands for CXCR1 and CXCR2 were described (Table 1) [2,17–21]. The tripeptide *N*-acetyl proline–glycine–proline (PGP), an extracellular matrix breakdown product, with structural homology to ELR+ chemokines, was described to bind and activate both CXCR1 and CXCR2 on human polymorphonuclear leukocytes and was also chemotactic for murine neutrophils [17,18]. Furthermore, macrophage migration inhibitory factor, a well-known proinflammatory cytokine, has been described as a noncognate ligand of CXCR2, contributing to leukocyte recruitment and activation, as seen in atherosclerosis [19–21]. The murine homologs of CXCR1 and CXCR2 and chemokines to which they bind are cited on Table 1.

Several studies have shown that CXCL8 can be released by a wide variety of cells after appropriate stimulation including monocytes [6,7,22], endothelial cells [23,24], T lymphocytes [25,26], fibroblasts [27], tumor cells [28], epithelial cells, hepatocytes, macrophages and synovial cells [29] and keratinocytes [30]. In their turn, the two known CXCL8 receptors, CXCR1 and CXCR2, are expressed on a wide variety of leukocytes, including neutrophils [6–8], monocytes [31], $CD8^+$ T cells [32,33], mast cells [34], basophils [35], natural killer cells [36] and myeloid-derived suppressor cells (MDSCs) [37]. In leukocytes, activation of either receptor induces chemotaxis and calcium flux. In neutrophils, receptor activation also stimulates the release of granule enzymes and generation of reactive oxygen species [38–40]. Cells other than leukocytes also expresses CXCR1 or CXCR2; these include keratinocytes [41], fibroblasts [42], neurons [43], endothelial cells [44], epithelial cells [45], smooth muscle cells [46], hepathocytes [47] and melanocytes (Table 2) [48]. In these cells, activation of the receptors may contribute to many actions including angiogenesis and consequent tumor growth [44,49–51]. Indeed, there is much evidence that CXCL8 and IL-8 family chemokines induce endothelial cell chemotaxis *in vitro* and angiogenesis *in vivo* [44,49–52].

CXCL8 family & receptors: from physiopathology to drug design

G protein-coupled receptors, such as CXCR1 and CXCR2, are proven targets for small molecule antagonists, and therefore, attractive targets for the development of novel therapies for inflammatory diseases. Selective antibodies, antagonists for CXCR1 and CXCR2 and gene-deficient mice exist, which allow the study of the function of the CXCL8 family of chemokines and their receptors. These tools together with data from human cells and samples have been useful to evaluate the role of the CXCL8 family in the context of various human diseases, as described below.

Atherosclerosis

Atherosclerosis is a chronic vascular inflammatory disease closely related to the metabolic syndrome. Atherosclerosis is characterized by cellular infiltration, mainly macrophages and lymphocytes, within atherosclerotic lesions, local proinflammatory cytokine production, matrix degradation and thrombosis [53,54]. Increased expression of CXCR2-binding chemokines

has been found in peripheral mononuclear cells and within atherosclerotic plaques in both humans [55,56] and mice [57,58]. Monocyte-derived macrophages are key players in atherogenesis because of their properties to form foam cells and due to the oxidation of lipoproteins aggregated to the intimal layer of blood vessels [59]. Once in contact with oxidized low-density lipoprotein, monocytes increase CXCR2 expression on their surface and are responsive for chemotaxis and adhesion under CXCL8 stimulation [53]. 25-hydroxycholesterol, which is an oxidized derivative of cholesterol, is also involved in the pathogenesis of atherosclerosis. Acting on retinoic-inducible gene I expressed on macrophages, 25-hydroxycholesterol induced the production of CXCL8 via activation of interferon regulatory factor 1 [60]. Furthermore, tissue factor and factor VIIa, which are also present within atherosclerotic lesions, contribute to vascular inflammation via stimulation of CXCL8 from endothelial cells [61]. In mouse studies, neutrophils seem to contribute to the pathogenesis of the disease, as shown by studies, which have described CXCR2-dependent neutrophils in large arteries and contribution of these cells to early phase of atherosclerotic plaque formation [62]. In another study, a modified recombinant protein of CXCL8 (G31P), which acts as an antagonist of CXCR1 and CXCR2, was used to treat atherosclerosis in high-fat diet-fed mice. The weekly treatment with G31P prevented the development atherosclerosis, decreased proinflammatory cytokine and metalloproteinases production in aorta and significantly reduced serum cholesterol levels, showing the importance of CXCR1/2 for atherosclerosis [63].

Table 2. Cell expression of agonists and chemokine receptors from IL-8 family.

Leukocyte population	Chemokine expression	Receptor expression
Neutrophils	CXCL1/GRO-α, CXCL2/GRO-β	CXCR1/CXCR2
Monocytes	CXCL1/GRO-α, CXCL8	CXCR1/CXCR2
T cells $CD8^+$	CXCL8	CXCR1
Mast cells		CXCR1/CXCR2
Basophils		CXCR1/CXCR2
Natural killer cells		CXCR1/CXCR2
Myeloid-derived suppressor cells		CXCR1/CXCR2
Macrophages	CXCL5/ENA-78, CXCL8	
T cells	CXCL1/GRO-α, CXCL2/GRO-β, CXCL3/GRO-γ	CXCR1/CXCR2
Eosinophils	CXCL1/GRO-α, CXCL5/ENA-78	
Nonleukocyte population	**CXC chemokine expression**	**Receptor expression**
Keratinocytes	CXCL8	CXCR1/CXCR2
Fibroblasts	CXL5/ENA-78, CXCL8	CXCR1/CXCR2
Epithelial cells	CXCL1/GRO-α, CXCL5/ENA-78, CXCL8	CXCR1/CXCR2
Hepatocytes	CXCL8	CXCR1/CXCR2
Synovial cells	CXCL8	
Endothelial cells	CXCL1/GRO-α, CXCL3/GRO-γ, CXCL5/ENA-78, CXCL6/GCP-2, CXCL8	CXCR1/CXCR2
Neurons		CXCR1/CXCR2
Melanocytes		CXCR1/CXCR2
Smooth muscle cells	CXCL1/GRO-α, CXCL2/GRO-β, CXCL3/GRO-γ	CXCR1/CXCR2
Tumor cells	CXCL1/GRO-α, CXCL2/GRO-β, CXCL3/GRO-γ, CXCL8	CXCR2
Oligodendrocytes	CXCL1/GRO-α	CXCR2
Trophoblasts	CXCL2/GRO-β, CXCL3/GRO-γ, CXCL6/GCP-2, CXCL8	
Endometrial cells	CXCL2/GRO-β	

Statins are drugs commonly used by hypercholesterolemic patients to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase. Hol and collaborators investigated another function for statins that may be relevant for the treatment of atherosclerosis. In Human umbilical vein endothelial cells, atorvastatin was able to retain CXCL1 and CXCL8 in endothelial E-selectin-containing endosomes and CD63-positive multivesicular bodies, suggesting that these chemokines can be destined for lysosomal degradation [64]. Altogether, these results show that CXCL8 is produced during atherosclerosis and may act on multiple cellular targets to facilitate disease progression including neutrophils, macrophages and endothelial cells. Experimental blockade of CXCL8 or its receptors prevents disease progression, suggesting that the pathway may be a potential target for the treatment of atherosclerosis and related conditions.

Cancer

The CXCL8 family of chemokines promotes several activities relevant to cancer including angiogenic responses in endothelial cells, increased proliferation and survival of endothelial and cancer cells and induction of the migration of cancer cells, endothelial cells and infiltrating neutrophils at the tumor site [65]. CXCL8 has been suggested to be a growth factor for certain tumoral cells. Indeed, CXCL1–3 is expressed in human

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melanoma and may mediate tumorigenesis of melanona via stimulation of angiogenesis [66]. The progression and growth of ovarian carcinoma are also dependent on angiogenesis, and CXCL8 is suggested to be an enhancer of human ovarian carcinoma tumorigenesis by an effect on neovascularization [67]; high expressing CXCL8 cells caused increased lethality when implanted into the peritoneum of mice. These studies have been extended to a lung cancer syngeneic tumor model system in mice. Lung cancer demonstrated angiogenesis-dependent reduced growth, increased tumor necrosis and reduced metastatic potential in CXCR2 knockout (KO) mice [68,69]. Prostate cancer tumorigenesis and metastases are also dependent on angiogenesis for progression in patients [70,71]. Serum levels of CXCL8 have been found to be markedly elevated in patients with prostate cancer and human prostate cancer cells constitutively producing angiogenic CXCL8 [72]. Depletion of endogenous CXCL8 inhibited prostate cancer tumor growth in severe combined immunodeficiecy mice that were entirely attributable to inhibition of tumor-derived angiogenesis [72]. The overexpression of CXCL8 enhanced the tumorigenicity and metastatic potential of melanoma cells *in vivo* [73]. CXCL8-transfected cells displayed upregulation of metalloproteinase-2, and this expression was accompanied with increased collagenase activity and increased invasiveness *in vitro* [73]. A close correlation of CXCL8 expression and vasculogenesis and metastasis is also shown in other malignancies including gastric carcinoma [74], breast cancer [75], melanoma [76] and head-and-neck cancer [77].

ACKR1/DARC binds angiogenic CXC chemokines (Table 1). Stable transfection and overexpression of ACKR1 in a nonsmall-cell lung carcinoma tumor cell line resulted in the binding of the angiogenic ELR+ CXC chemokines produced by the tumor cells. This binding in turn resulted in decreased ability of angiogenic factors to stimulate endothelial cells and promote tumor-associated angiogenesis [78]. CXCR2 is the putative receptor that mediates angiogenic activity of ELR+ CXC chemokines. In agreement with the latter suggestion, aberrant expression of a homolog of CXCR2 was associated with cellular transformation relevant to preneoplastic to neoplastic transformation [79]. Indeed, Kaposi's sarcoma herpes virus is a homologous to CXCR2 and mediates the pathogenesis of Kaposi's sarcoma by supporting preneoplastic to neoplastic cellular transformation [79–81].

Tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs) can control cancer growth and are found in most solid tumors. The cells are known to descend from immature monocytic and granulocytic cells, respectively, which are produced in the bone marrow (BM) and spleen [82]. Indeed, monocytes treated with IL-4 and IL-13 expressed CXCR1 and CXCR2 receptors with characteristics of alternative macrophage polarization (M2) [31] and contributed to mediating peritoneal invasion and metastasis of epithelial ovarian cancer [83]. It has been recently demonstrated that macrophages isolated from the tumor microenvironment of inflammatory breast cancer (IBC) patients secreted CXCL8 that significantly increased motility and invasion of IBC cells *in vitro*, an effect that may contribute to dissemination and metastasis of IBC in humans [84]. Myeloid angiogenic cells are monocytic cells without endothelial characteristics and represent alternative activated M2 macrophages with proangiogenic and protissue repair activities induced by CXCL8 secretion [85].

Intratumoral CXCL8 expression is suggested to be a key regulator of the recruitment of neutrophils into the tumor microenvironment, the potential consequence of which is the promotion of metastasis [86]. An interesting unanswered question is how the phenotype of TAN is influenced by the ongoing evolvement of tumor microenvironment. Recently, it has been shown that TANs can have antitumorigenic (N1) or protumorigenic (N2) functions [87,88]. TGF-β blockade also increases neutrophil-attracting chemokines, resulting in an influx of $CD11b^+/Ly6G^+$ TANs that are hypersegmented, more cytotoxic to tumor cells and express higher levels of proinflammatory cytokines and are antitumorigenic (N1) [88]. The depletion of these neutrophils significantly blunted antitumor effects and reduced $CD8^+$ T cell activation. TGF-β functions in the tumor microenvironment may be maintaining a population of TANs with a protumor phenotype [88], and intratumoral neutrophil depletion decreases tumor growth. On the other hand, TANs are recruited by CXCL2 produced by TAMs [89]. These neutrophils express metalloproteinase-9 and neutrophil elastase and contribute to angiogenesis and cancer proliferation [89]. In agreement with the latter suggestions, TANs may play a role in the initiation and progression of colitis-associated colon cancer and suggest that the CXCL2–CXCR2 axis might be useful in reducing the risk of ulcerative colitis (UC)-associated colon cancer [89]. The chemokine receptor CXCR2 appears to be the most relevant receptor for homing of TANs into developing tumors sites.

Tumor growth is associated with aberrant myelopoiesis including the accumulation of CD11b+GR-1+ MDSCs that have the potential to promote tumor growth. Genetic evidence supports that the loss of CXCR2 suppresses the chronic–colonic inflammation and colitis-associated tumorigenesis by inhibiting infiltration of MDSCs into colonic mucosa and tumors in a mouse model of colitis-associated cancer. CXCR2 ligands were elevated in inflamed colonic mucosa and tumors, and induced MDSC chemotaxis, accelerating the tumor growth by inhibiting $CD8^+$ T cell cytotoxic activity [37]. The serum levels of CXCL8 were found elevated in patients with kidney cancer. Kidney cancer cells also secreted CXCL8 that activated the Akt signaling pathway through CXCR2 receptor expressed by myeloid stem cells (MSCs), inducing the migration of MSCs. Blocking CXCL8 or CXCR2 impaired the migration ability of MSCs *in vitro* [90]. CXCL8 is reported to promote breast cancer progression by increasing cell invasion, angiogenesis and metastases and is also involved in regulating breast cancer stem-like cells recruitment and activity [91]. The mobilization of BM-derived endothelial progenitor cells (EPCs) that promote angiogenesis is dependent of CXCR2 in a model of pancreatic cancers. The circulating levels of EPCs are decreased in the BM and/or blood of tumor-bearing CXCR2 KO mice.

Moreover, CXCR2 gene KO reduced BM-derived EPC proliferation, differentiation and vasculogenesis *in vitro* [92], indicating a role of BM-derived EPC in pancreatic cancer growth and via CXCR2-mediated tumor neovascularization.

Collectively, these data suggest that CXCL8 family of chemokines and receptors is interesting targets for modulation of TAMs and TANs infiltration, tumor stem cells recruitment, angiogenesis and metastasis in cancer-related malignancies.

Inflammatory bowel disease

Crohn's disease (CD) and UC are the main chronic inflammatory bowel diseases (IBD) and characterized by chronic relapsing inflammation of the gastrointestinal tract. Genetic and environment factors contribute to the development of IBD, and mechanisms that trigger disease are directly correlated to dysregulation of the immune response to intestinal microbiota, leading to common symptoms such as diarrhea, abdominal pain, rectal bleeding and malnutrition [93]. In noninflamed human colon, CXCR1 is expressed in macrophages deep in the epithelium and germinal center lymphocytes, while this receptor is upregulated in macrophages and luminal epithelium in CD, suggesting that these cells have participation on intestinal homeostasis and also during CD [94,95]. Inflamed epithelial cells from IBD patients can secrete several chemokines including CXCL8 [96]. Neutrophils migrated into the epithelium and lamina propria in response to this chemokine may cause important mucosal damage via release of metalloproteinases, reactive oxygen species and cytokines. The signaling of CXCR2 by CXCL2 activates STAT3, and this contributes to neutrophil migration [97]. Polymorphic analysis of potential genes in patients with pediatric CD demonstrated that mutations of *stat3* gene are associated to increase STAT3 activation and interfere in the pathway for neutrophil CXCR2+ migration, which accumulate in the gut of these patients, amplifying inflammation [98]. Consistently, with these studies in humans, inhibition of CXCR2 has been demonstrated to improve intestinal inflammation in various experimental models of IBD [99–102].

However, CXCR2-dependent neutrophil migration may be a relevant mechanism to control microbial spreading after infection and even in the context of IBD, as exemplified here in mouse studies. For example, following *Citrobacter rodentium* infection in mice, which causes intense intestinal inflammation and diarrhea, the absence of CXCR2 or decreased levels of CXCL1 reduced neutrophil infiltration in lamina propria. This was associated with increased bacterial detection in feces and intense diarrhea when compared with wild-type (WT) mice [103,104]. The cytokine IL-22 is protective in experimental models of colitis [105]. In dextran sodium sulfate (DSS)-induced model of acute colitis in mice, neutrophils infiltrated in inflamed tissue are an important source of IL-22, and this cytokine is associated with an increase of epithelial cell-derived antimicrobial peptides, S100A8 and S100A9 [105]. Thus, lack of CXCR2-driven neutrophil influx may decrease this IL-22-dependent protective mechanism.

Chronic inflammation of the gastrointestinal tract, such as it occurs during IBD, can progress to cancer. The continuous infiltration of leukocytes in the latter scenario is believed to be pivotal for tumorigenesis [106]. Experimentally, in DSS-induced adenomas, there is an increase of CXCR2-activating chemokines produced in tumor tissue with a high number of migrated neutrophils. In this case, mice deficient for CXCR2 not only had improved clinical aspects of colon inflammation but also had suppressed adenoma formation [107]. Consistently, CXCL8 is overexpressed in human colorectal tumors [108]. Using a similar DSS model, researchers demonstrated that mice expressing a human gene for CXCL8 developed more tumors when compared with WT mice, and this was associated with higher number of CD11b+GR1+ immature myeloid cells which are an important source of more CXCL8, promoting angiogenesis and tumor growth [108]. Therefore, there is much experimental and clinical evidence for the protumorigenic effects of CXCL8 and its receptors in the context of IBD. Part of these effects may be secondary to the action of the chemokine on neutrophils, but effects on endothelial and potentially on other cell types could also contribute to tumorigenesis.

Infection

Although excessive neutrophil recruitment is harmful in many pathological conditions, as described above, these cells are thought to play an important role in pathogen clearance, especially bacteria and fungi. Studies performed in different models, mostly in mice, have shown that the absence or blockade of CXCR2 leads to reduced neutrophil infiltration into the infection site, but increased burden of *Streptococcus pneumoniae* [109], *Aspergillus fumigatus* [110], *Toxoplasma gondii* [111] and *Yersinia pestis* [112]. The role of the putative murine CXCR1 has not been studied in detail in mice. CXCR2 absence or antagonism using SB-225002 caused reduction not only in neutrophil influx but also in exudate macrophages, leading to reduced *S. pneumoniae* elimination and increased lethality [109]. The impaired host defense against *S. pneumoniae* was observed even when CXCR2 ablation was partial, through chimeric WT mice reconstitution with successively increased amounts of CXCR2-deleted hematopoietic cells [109]. Neutrophils also play an important role in host defense against *A. fumigatus* and antibody neutralization of CXCR2 in infected immunocompetent mice resulted in an invasive disease with reduced lung neutrophil numbers and enhanced lethality, whereas CXCL1 overexpression protected neutrophil-depleted mice from lethality [110]. CXCR2-deficient mice were also more susceptible to *A. fumigatus* challenge if nonsensitized and had 50% lethality in 3 days, whereas WT mice did not succumb to the challenge with *A. fumigatus* conidia [113]. Overall, studies with various bacteria and fungi do suggest an important role for CXCR2, especially on neutrophils, for host resistance against infection. These experiments in animals suggest that close follow-up of patients treated with blockers of the CXCL8 family is necessary to evaluate safety.

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Neutrophils are not considered the major effector cell against viral infection. However, there is evidence to suggest that CXCR2 might contribute to disease control after infection. For example, in mouse hepatitis virus, neutrophil infiltration into the CNS contributes to blood–brain barrier permeabilization. CXCR2 neutralization reduced accumulation of virus-specific T cells within the CNS, increasing mortality and viral replication [114]. On the other hand, there is evidence to suggest that neutrophils contribute to disease progression. For example, cytomegalovirus has an interesting mechanism to increase replication, that is, the presence of the UL146 gene that encodes a CXC chemokine which acts as an agonist of CXCR1 and CXCR2 and might stimulate neutrophils to carry the virus to uninfected cells; in this case, CXCR1 and CXCR2 antagonists might represent an important approach to reduce viral infection and pathology [115]. In a mouse model of human Rhinovirus infection, it was shown that neutrophil depletion or absence of CXCR2 results in reduced airway and lung infiltration of neutrophils and cholinergic hyperresponsiveness [116].

Sepsis

Sepsis is a complex syndrome associated with the host response to microbes [117]. The deleterious effects of clinical sepsis are consequence of microbial products and uncontrolled inflammatory mediators release by cell activation, leading to tissue damage and organ dysfunction [118]. Neutrophils are crucial cells to deal with infection since they are quickly recruited from the bloodstream and have an arsenal to combat the infectious microbes via releasing a vast γ of mediators [119,120]. Several studies have evaluated the role of the chemokine receptor CXCR2 and its ligands and their importance for neutrophil recruitment in order to control microbial dissemination in sepsis. CXCL8 is produced in sepsis, and its detection in serum could be a prediction for its severity and evolution to organ dysfunction [118].

Studies *in vitro* and *in vivo* have demonstrated that endothelial cells recognize microbial products and release substantial amounts of chemokines that bind to CXCR2 [121–123]. This process is important for neutrophil migration to infected tissue in order to combat the infection. However, massive CXCR2 activation by their agonists desensitizes its receptor, leading to failure of further neutrophil migration and culminating in microbial dissemination, uncontrolled systemic inflammation and death of patients [124,125]. The mechanisms of CXCR2 desensitization and the molecules involved in this process in sepsis have been studied in experimental models. The overstimulation of neutrophils by these mediators leads to increase of intracellular GRK2 (GPCR kinase 2) expression, which phosphorilates the GPCR, a step for CXCR2 internalization [126]. Moreover, other mediators that are upregulated in large scale in severe sepsis, such as TNF-α, heme oxygenase and iNOS activity also contribute to this phenomenon [125,127,128]. Interestingly, CXCR1 persists on the surface of neutrophils after sepsis in humans, probably via a rapid re-expression on the surface [124,129].

Based on the role of neutrophils in controlling infection and the observation that failure of neutrophils to migrate to the site of infection associates with sepsis severity, strategies that prevent failure of neutrophils to migrate are thought to be useful in the context of sepsis. PI3Kγ is an enzyme involved in leukocyte activation and motility, which can be activated after GPCR–agonist interaction including CXCR2 and its ligands [130]. The absence or blockade of PI3Kγ in a mice model of sepsis (CLP, cecal ligation and puncture) reduced the susceptibility to neutrophil migration failure, leading to better control of systemic inflammation. This process was associated with a reduced expression of GRK2 and consequently keeps sufficient levels of CXCR2 on surface of neutrophils for their migration to the focus of infection [131]. In another study, the administration of the cytokine IL-33 in septic mice (CLP model) was effective to keep neutrophil migration to peritoneal cavity (focus of infection), allowing them to deal with microbes and decrease death [132]. The mechanism of action was also associated with inhibition of GRK2 protein. Moreover, this study demonstrated that patients who did not recover from sepsis had an increase of soluble ST2 (the decoy receptor for IL-33), showing that IL-33 has a potential option for therapeutic in sepsis [132].

Neutrophil recruitment is not only dependent on the CXC chemokines and CXCR2 in the context of sepsis. CCR2 is expressed on neutrophil surface, and its ligands are produced following different models of sepsis in mice [133–135]. CCR2-deficient mice presented reduced lung injury and lethality compared with WT mice, and the deleterious effect of accumulated neutrophils in lungs was reduced due to the diminished recruitment to this organ [135]. However, this inhibition impaired the bacterial clearance, which represents a risk for severe systemic sepsis [133,135], demonstrated how important must be the control of chemokine receptor in this condition. Thus, a fine-tune control of CXCR2 or CCR2 expression on neutrophil must be well controlled to avoid excessive or failure of neutrophil recruitment, which directly implicate in the control of infection and inflammation. Overall, the case for CXCL8 family members in the context of sepsis is beneficial.

Ischemia & reperfusion injury

The interruption of tissue blood flow is directly correlated to the pathophysiology of several diseases including stroke, hypovolemic shock and myocardial infarction. Ischemia and reperfusion (I/R) are also unavoidable after certain surgical procedures such as organ transplantation and coronary angioplasty [136]. Apart from the lesion caused by ischemia, especially the damage of the microvasculature, the necessary restoration of blood flow (reperfusion) can aggravate the injury initialized on local organ and potentially reach remote organs, developing the systemic inflammatory response and multiple organ dysfunction syndromes [136]. Endothelial and parenchyma cells together with various leukocytes are highly sensitive to the deleterious effect of I/R becoming activated and producing a range of inflammatory mediators. Among them, CXCL8 and other

CXCR1/2 ligands are upregulated in the absence of oxygen, and their production is directly associated with the time of reperfusion in both human cells [137] and mice [138].

Infiltrated neutrophils can exacerbate the inflammatory response via releasing several mediators including reactive oxygen species, proteases and cytokines [138,139] and the inhibition of their migration could be an alternative strategy to avoid the tissue damage caused by I/R. In agreement with this possibility, studies have demonstrated the beneficial effect of the blockage of CXCR2 or the inhibition of its ligands in different models of I/R [138,140]. Regarding the inflammatory response originated from an ischemic organ, a systemic inflammation frequently occurs and may potentially cause the death of the affected individual [141]. Several inflammatory mediators including TNF-α can be found in high amounts in serum and lung after intestinal I/R injury [138]. Therapeutic strategies that block CXCR2 or inhibit the action of its ligands have been shown to decrease local and systemic inflammation [138].

CXCR2-expressing cells, other than neutrophils, may also contribute to the pathogenesis of I/R injury. Brain ischemia can stimulate the expression of CXCR2 on microglial cells, which are a source of CXCR2 ligands, a fact that is associated with brain injury [142]. In another study, the neutrophilic recruitment to the liver was similar in both WT and CXCR2-deficient mice late after reperfusion, and the blockade of CXCR2 at this point was beneficial for liver regeneration, showing that CXCR2 signaling on hepatocyte has a negative impact in this system [143]. In another study using chimeric mice, the main deleterious effect of CXCR2 signaling in hepatic I/R was on leukocytes, with a secondary role on hepatocytes [144]. Altogether, the main body of evidence suggests that activation of CXCR1/2 by the CXCL8 family of chemokines contributes to tissue and systemic damage after IR. Blockade of this chemokine system prevents disease and may contribute to spare tissue. This should be exploited clinically and may also bear relevance to transplantation.

Lung diseases

Acute lung injury

Acute lung injury (ALI) results from direct injury to the lung by conditions such as bacterial pneumonia, aspiration or noxious substances inhalation, trauma, but also from indirect injury as a result of a systemic inflammatory disease such as sepsis, pancreatitis and blood transfusion [145]. ALI causes high morbidity and mortality and might evolve to a more severe manifestation called acute respiratory distress syndrome (ARDS). During ALI, inflammation results in increased vascular permeability and matrix remodeling, pulmonary edema and impaired gas exchange that lead to hypoxemia [145–147]. The degree of neutrophil accumulation in the lungs of ALI following sepsis patients correlates with disease severity and mortality [148]. The levels of mediators G-CSF, CXCL5, CXCL8 correlate with neutrophil numbers and were found in higher levels in ARDS [149–151]. Besides increased CXCL8 levels, patients at risk for ARDS presented increased anti-CXCL8 autoantibodies [152] and the presence of these antibodies in immune complexes with CXCL8-enhanced survival of neutrophils via the IgG receptor FcγRIIa, and this is a possible mechanism for enhanced neutrophil accumulation during ALI [153]. CXCL5 is also important for neutrophil recruitment after LPS stimulation but only from lung parenchyma to airways [154]. Genetic factors might contribute to ALI susceptibility. For example, a polymorphism on ACKR1 gene was shown to be associated to ALI mortality [155], an effect that could be due to the capacity of ACKR1 to keep the homeostasis of CXCL1 with its receptor CXCR2 during ALI [156].

CXCR1 and CXCR2 ligands have been demonstrated to mediate ALI in different experimental models including acid aspiration [157], acute pancreatitis associated lung injury [158], ventilator-induced lung injury (VILI) [159], hyperoxia-induced lung injury [160] and LPS inhalation [157,161]. In the acid aspiration model, treatment with the CXCR2 inhibitor reparixin-reduced intravascular, interstitial and airways neutrophil recruitment, vascular permeability and improved gas exchange [157]. In a pancreatitis model, mice treated with anti-leukinate, a CXCR2 antagonist, were protected from pancreatitis and from associated lung injury [158]. In the VILI model, the blockade of CXCL1, CXCL2 or CXCR2 with specific antibodies attenuated VILI, neutrophilic infiltration and vascular permeability. CXCR2 KO mice were also protected from VILI manifestations [159]. During hyperoxia-induced lung injury, neutrophil recruitment in the lungs is accompanied by enhanced CXCR2, CXCL1 and CXCL2 expression; CXCR2 KO mice submitted to hyperoxia were protected from lethality, showing reduced lung injury and neutrophil infiltration [160]. The LPS model of ALI is characterized by a huge infiltration of neutrophils in the lungs and airways and also vascular leakage; in the absence of CXCR2, LPS-challenged mice presented reduction in both neutrophil accumulation and vascular leakage [161]. BM chimeras using donors and recipient WT or CXCR2 KO mice revealed that endothelial and epithelial cells also express CXCR2, and the expression of the receptor in these cells contributes to effective neutrophil recruitment [161]. In the same model, reparixin reduced in a dose-dependent way the neutrophil accumulation in the airways and interstitial but not intravascular neutrophil numbers and vascular permeability [157].

Results from three clinical trials evaluating CXCR2 antagonists in ALI have been published [162–164]. SB656933 [162] and SCH527123 [163] were tested in health volunteers subjected to ozone-induced neutrophilia. The treatment reduced neutrophils numbers in sputum and in blood [162,163] and reduction in CD11b+ expression by peripheral blood neutrophils [162]. AZD 8309 was also tested in healthy volunteers challenged with LPS [164]. Treatment with AZD 8309 reduced neutrophil counts, elastase activity and Leukotriene B4 levels [164]. The vast amount of data in experimental models of ALI and in clinical trials reinforce the importance of CXCR1 and CXCR2 signaling to cause lung damage by mediating neutrophil recruitment and vascular permeability after a direct injury or a remote damage to a systemic inflammatory response.

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Asthma

Asthma is a chronic inflammatory eosinophilic respiratory disease characterized by thickening of airway walls and associated with recurrent airflow obstruction and hyperresponsiveness [165]. Patients undergoing severe asthma attacks are referred to be in *status asthmaticus* and present increased neutrophilia, eosinophilia, CXCL8, neutrophil elastase and eosinophil cationic protein levels compared with normal asthmatic or healthy donors, which might reflect more tissue damage [166]. The chemokines CCL2, CCL3 and CXCL8 were found elevated in the sputum of asthmatic patients before asthma attacks and might be related to late-phase exacerbation of the disease [167]. CXCL5 and CXCR2 are more frequent in patients with severe exacerbation of asthma and correlate with eosinophil numbers [168]. CXCL8 might contribute to asthma pathology, enhancing neutrophilic pulmonary infiltration and lung damage [166] or by direct bronchoconstrictor effects on human airway smooth muscle (ASM) cells [169], which do express CXCR1 and CXCR2 [170]. CXCL2 and CXCL3 could also bind to CXCR1 on human ASM cells and induce migration of the cells, a process related to the increase in the numbers of these cells in asthma [46].

Different experimental airway inflammation models that mimic asthma manifestations evaluated the role of CXCR2 in disease pathogenesis [113,116,171,172]. CXCR2 KO OVA-challenged mice presented reduced neutrophil numbers but increased B cells and OVA-specific IgE levels, but no histological changes in the lungs [171]. In *A. fumigatus*-induced asthma, CXCR2 KO mice were protected from disease manifestations like chronic airway hyperresponsiveness, peribronchial and airway changes, reduced eosinophil and T cell migration, IL-4, IL-5, CCL5 and CCL11 [113]. Besides the role of CXCR2 on leukocyte chemotaxis during asthma, the chemokine receptor is also involved in the migration of EPCs [172]. During OVA-induced airway inflammation, blockade of CXCR2 reduces EPC recruitment and lung neovascularization, an event related to asthma pathology [172].

Based on the possible role of CXCR2 in different aspects of asthma pathology – neutrophil infiltration, IgE production, bronchoconstriction, ASM cells and EPCs migration – four clinical trials have tested CXCR2 antagonist in asthmatic patients (Table 3). There is reported data for compound SCH527123, which reduced neutrophilic infiltration in asthmatic patients who evolved with milder exacerbations [173]. Therefore, blockade of CXCR1/2 in the context of acute exacerbations of asthma may be useful, as suggested by experimental studies and initial clinical trials. There are less data to suggest that these compounds may be useful in chronic asthma.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD), a disease caused primarily by cigarette smoke exposure, which causes an acute inflammatory response, oxidative stress, cell death and proteolysis of extracellular matrix [174]. The disease progresses to chronic bronchitis and emphysema that limit airflow and might be exacerbated by bacterial and viral infections [174]. Cells from COPD patients present increased chemotatic response to CXCL1, CXCL7, but not CCL2, CXCL5 or CXCL8, and this chemotaxis was inhibited by treating cells with the CXCR1 and CXCR2 antagonist SB468477 [175]. Moreover, COPD patients present elevated levels of *N*-Acetyl PGP, a neutrophil chemoattractant released by extracellular matrix degradation that binds to CXCR1 and CXCR2 [17]. Mice instilled with PGP presented alveolar enlargement similarly to mice exposed to cigarette smoke [17].

In a COPD model using cigarette exposure to rats, acute neutrophil and globet cell accumulation and mucin production were observed in a cigarette dose-dependent way, and this was related to increased levels of the chemokines CXCL1, CXCL2 and CXCL3 [176]. Treatment with the CXCR2 antagonist SB-332235 reduced neutrophilic infiltration in airways and lungs and CXCL3 production and globet cell density [176]. In a similar model of cigarette exposure in mice, treatment with the CXCR2 antagonist SCH-N (now known as SCH527123) also reduces neutrophil infiltrations in the airways and lungs and reduced β-glucuronidase activity as well, an indication of reduced tissue damage [177]. All of these studies suggest the involvement of CXCL8 family members in the pathogenesis of COPD. Indeed, many of the current clinical trials with CXCR1 or CXCR2 antagonists or anti-CXCL8 (Table 3) have focused on COPD, but reports are still not available.

Cystic fibrosis

Cystic fibrosis (CF) results from the presence of two mutant alleles of the CF transmembrane conductance regulator gene that encodes an anion channel expressed in epithelial cells. The disease affects many organs, especially the pancreas and lungs. The leading cause of CF death is respiratory failure that results from a combination of chronic obstruction of airways, neutrophilic inflammation and bacterial infections [178]. There are high levels of CXCL8 in sputum of CF patients, and these cause significant chemotaxis of neutrophils in an *in vitro* system [179]. CXCL8 exposure in culture of ASM cells causes increased contraction in CF ASM cells than in healthy controls, which might contribute to airway hyperresponsiveness [180]. A screening of a SNP on CXCR1 and CXCR2 genes in CF patients showed that the presence of specific SNPs correlated to lung function and antibacterial functions [181]. It has been suggested that CXCL8 mediates antibacterial activities in neutrophils by activating CXCR1, but not CXCR2. CXCR1 levels on neutrophils are reduced in CF patients and correlate to decreased antibacterial activity. CXCR1 cleavage by elastases and cathepsin G during CF releases active peptides that activate TLR2, inducing CXCL8 production, which can exacerbate inflammatory response during CF [182]. Another study showed that neutrophil extracellular traps (NETs), an important mechanism for bacterial clearance but also related to tissue damage, are present in sputum, bronchoalveolar lavage fluid and lung tissue of CF patients and correlate with poorer lung function and CXCL2 levels [183]. CXCR2 blockage with SB225002 in

Table 3. Available drugs and clinical trials.

Compound	Condition	Strategy	Company	Trial number	Title	Phase	Ref.
ABX-IL8	COPD, Bronchitis	Anti-CXCL8 monoclonal antibody	Abgenix	NCT00035828	A blinded study comparing the safety and efficacy of a fully human anti-IL8 monoclonal antibody (ABX-IL8) to placebo in patients with chronic bronchitis and COPD	Phase II: completed	–
AZD 5069	Asthma	CXCR2 antagonist	AstraZeneca	NCT01890148	Distribution of neutrophils in bronchial mucosal tissue in asthma patients before and after 4 weeks treatment with AZD 5069	Phase II	–
AZD 5069	Healthy subjects	CXCR2 antagonist	AstraZeneca	NCT01480739	AZD 5069 Neutrophil Function Study	Phase I: completed	–
AZD 5069	Healthy subjects	CXCR2 antagonist	AstraZeneca	NCT01735240	Phase I study to assess the effect on healthy male volunteers of ketoconazole on the PK of a single dose of AZD 5069 administered orally	Phase I: completed	–
AZD 5069	Asthma	CXCR2 antagonist	AstraZeneca	NCT01704495	A Phase II study to evaluate the efficacy, safety and tolerability of AZD 5069 in patients with uncontrolled persistent asthma (NIMBUS)	Phase II: ongoing	–
AZD 5069	Healthy subjects	CXCR2 antagonist	AstraZeneca	NCT01332903	Open label, healthy volunteers, ADME study with single-oral administration of (14C) AZD 5069	Phase I: completed	–
AZD 5069	Healthy subjects	CXCR2 antagonist	AstraZeneca	NCT00953888	Study to investigate the safety, tolerability and activity of AZD 5069 when given as a single dose to healthy male and/or female subjects	Phase I: completed	–
AZD 5069	Healthy subjects	CXCR2 antagonist	AstraZeneca	NCT01083238	This study will investigate how food and age affect the way the body handles the AZD 5069 drug given as a oral dose	Phase I: completed	–
AZD 5069	COPD	CXCR2 antagonist	AstraZeneca	NCT01962935	Study to investigate safety, tolerability and effect of multiple dosing with AZD 4721 and/or with AZD 5069	Phase I	–
AZD 5069	Healthy subjects	CXCR2 antagonist	AstraZeneca	NCT01051505	A study to assess the safety, tolerability, PK and PD of multiple ascending doses of AZD 5069 in healthy volunteers	Phase I: completed	–
AZD 5069	COPD Chronic bronchitis and emphysema	CXCR2 antagonist	AstraZeneca	NCT01233232	A 4-week study to investigate the safety and tolerability of AZD 5069 in patients with moderate-to-severe COPD (CIRRUS)	Phase II: completed	–

Word search in clinicaltrials.gov: ABX-IL8, AZD 5069, CXCL8, CXCR1, CXCR2, GSK1325756, IL-8, PA401, reparixin, SB656933, SCH 527123.
COPD: Chronic obstructive pulmonary disease; PD: Pharmacodynamics; PK: Pharmacokinetics.

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Table 3. Available drugs and clinical trials (cont.).

Compound	Condition	Strategy	Company	Trial number	Title	Phase	Ref.
AZD 5069	Healthy subjects	CXCR2 antagonist	AstraZeneca	NCT01100047	Japanese single and multiple ascending dose, safety, tolerability, PK and PD study of AZD 5069	Phase I: completed	–
AZD 5069	Bronchiectasis Lung disease Respiratory diseases	CXCR2 antagonist	AstraZeneca	NCT01255592	Evaluation of the effect of AZD 5069 in patients with bronchiectasis (STRATUS)	Phase II: completed	–
AZD 8309	Healthy subjects	CXCR2 antagonist	AstraZeneca	NCT00860821	A methodology study in healthy subjects to evaluate the effect of AZD 8309 after nasal administration of lipopolysaccharide	Completed	[164]
DF2156A	Bullous pemphigoid	CXCR1/2 antagonist	Dompé s.p.a.	NCT01571895	Pilot efficacy and safety study of oral DF2156A in patients with active bullous pemphigoid	Phase II: completed	–
GSK1325756	COPD	CXCR2 antagonist	GlaxoSmithKline	NCT01209052	First time in human study with GSK1325756	Phase I: completed	–
GSK1325756	COPD	CXCR2 antagonist	GlaxoSmithKline	NCT01453478	A study to look at how GSK1325756 is taken up by the body when given by mouth when stomach acid is reduced	Phase I: completed	–
GSK1325756	COPD, healthy volunteers	CXCR2 antagonist	GlaxoSmithKline	NCT01267006	Blood levels and effects of GSK1325756 in healthy adult volunteers aged 40–80 years old	Phase I: completed	–
GSK1325756	COPD, nutritional status	CXCR2 antagonist	GlaxoSmithKline	NCT01209104	PK and safety of GSK1325756 in elderly and adult subjects in the fed and fasted states and in the presence of a proton-pump inhibitors	Phase I: completed	–
HuMab 10F8	Palmoplantar pustulosis, healthy volunteers	Human IL-8 Anti-CXCL8 antibody					[281]
PA401	Healthy volunteers	Modified IL-8	ProtAffin biotechnologie AG	NCT01627002	A Phase I, first in human study to investigate the safety and tolerability of PA401	Phase I: completed	–
Reparixin	Breast cancer	CXCR1/2 antagonist	Dompé s.p.a.	NCT01861054	A pilot study to evaluate the safety and biological effects of orally administered reparixin in early breast cancer patients	Phase II: recruiting	–
Reparixin	Pancreatic islet transplantation in Type 1 diabetes mellitus	CXCR1/2 antagonist	Dompé s.p.a.	NCT01220856	Reparixin in pancreatic islet transplantation	Phase II: ongoing	[209]

Word search in clinicaltrials.gov: ABX-IL8, AZD 5069, CXCL8, CXCR1, CXCR2, GSK1325756, IL-8, PA401, reparixin, SB656933, SCH 527123.
COPD: Chronic obstructive pulmonary disease; PD: Pharmacodynamics; PK: Pharmacokinetics.

Table 3. Available drugs and clinical trials (cont.).

Compound	Condition	Strategy	Company	Trial number	Title	Phase	Ref.
Reparixin	Ischemia-reperfusion injury; kidney diseases	CXCR1/2 antagonist	Dompé s.p.a.	NCT00248040	Reparixin in prevention of delayed graft dysfunction after kidney transplantation	Phase II: completed	–
Reparixin	Islet transplantation in diabetes mellitus type 1	CXCR1/2 antagonist	Dompé s.p.a.	NCT01817959	Study to assess efficacy and safety of reparixin in pancreatic islet transplantation	Phase III: recruiting	–
Reparixin	Pancreatic islet autotransplantation in diabetes type 1	CXCR1/2 antagonist	Dompé s.p.a.	NCT01967888	Efficacy and safety of reparixin in pancreatic islet autotransplantation	Phase II Phase III: not open	–
Repertaxin	Ischemia-reperfusion injury; lung transplantation	CXCR1/2 antagonist	Dompé s.p.a.	NCT00224406	Repertaxin in prevention of primary graft dysfunction after lung transplantation	Phase II: completed	–
SB656933	COPD	CXCR2 antagonist	GlaxoSmithKline	NCT00504439	A study to evaluate the safety and tolerability of SB-656933-AAA, following repeated doses in healthy adult subjects	Phase I: completed	–
SB656933	Ulcerative colitis	CXCR2 antagonist	GlaxoSmithKline	NCT00748410	Study to evaluate the PD of SB-656933 in patients with ulcerative colitis	Phase II: completed	–
SB656933	COPD, cystic fibrosis	CXCR2 antagonist	GlaxoSmithKline	NCT00615576	Repeat dose study in male healthy volunteer smokers	Phase I: completed	–
SB656933	Cystic fibrosis	CXCR2 antagonist	GlaxoSmithKline	NCT00605761	SD cystic fibrosis study	Phase I: completed	–
SB656933-AAA	COPD, cystic fibrosis	CXCR2 antagonist	GlaxoSmithKline	NCT00551811	Evaluate the effects of the drug (SB-656933-AAA) on the body after a single dose in subjects who have inhaled ozone	Phase I: completed	[162]
SB656933	Cystic fibrosis	CXCR2 antagonist	GlaxoSmithKline	NCT00903201	28-day repeat dose in cystic fibrosis patients	Phase II: completed	[185]
SCH 527123	Asthma	CXCR1/2 antagonist	Merck	NCT00688467	Efficacy and safety of SCH 527123 in subjects with allergen-induced asthma (Study P05363)	Phase II: completed	[173]
SCH 527123	Psoriasis	CXCR1/2 antagonist	Merck	NCT00684593	A study to assess the clinical effects of SCH 527123 in psoriasis (study P04481 AM1)	Phase II: completed	–

Word search in clinicaltrials.gov: ABX-IL8, AZD 5069, CXCL8, CXCR1, CXCR2, GSK1325756, IL-8, PA401, reparixin, SB656933, SCH 527123.
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Table 3. Available drugs and clinical trials (cont.).

Compound	Condition	Strategy	Company	Trial number	Title	Phase	Ref.
SCH 527123	COPD	CXCR1/2 antagonist	Merck	NCT01068145	Two-part study to evaluate the dose response of SCH 527123 on sputum neutrophilia, following ozone challenge in healthy subjects and COPD patients	Phase I: completed	[163]
SCH 527123	COPD	CXCR1/2 antagonist	Merck	NCT00441701	Study to evaluate the safety and dose range of SCH 527123 in subjects with moderate-to-severe COPD (Study P04592AM4)	Phase II: completed	–
SCH 527123	Asthma	CXCR1/2 antagonist	Merck	NCT00632502	Neutrophilic asthma study with SCH 527123 (Study P05365AM2)	Phase II: completed	[173]
SCH 527123	COPD	CXCR1/2 antagonist	Merck	NCT01006616	Long-term extension study of the effects of SCH 527123 in subjects with moderate-to-severe COPD (P05575AM2)(MK-7123-009-0)	Phase II: completed	–

Word search in clinicaltrials.gov: ABX-IL8, AZD 5069, CXCL8, CXCR1, CXCR2, GSK1325756, IL-8, PA401, reparixin, SB656933, SCH 527123.
COPD: Chronic obstructive pulmonary disease; PD: Pharmacodynamics; PK: Pharmacokinetics.

βENaCTg mice, a model of neutrophilic CF-like lung disease, decreased NETs formation and improved lung function. However, cells obtained from different donors resulted in different effects on NETs formation [184]. PGP release after the cleavage of collagen by metalloproteases 8 and 9 and further cleavage of small collagen fragments by prolyl endopeptidase into PGP also activates CXCR1 and CXCR2 activation and neutrophil chemotaxis during CF [18].

The therapeutic potential of CXCR2 antagonism in CF was assessed using SB656933 that reduced neutrophil numbers and activation after 28 days of treatment (Table 3) [185]. These studies suggest that neutrophils driven by CXCR2 (in rodents) play an important role in the context of CF and contribute to pulmonary damage. In this regard, blockade of CXCR1/2 may be useful for the treatment of CF in humans. However, CXCR1/2-mediated neutrophil influx has been shown to contribute to the clearance of bacteria in various models of infection [109,186]. As bacterial infections are a common complication and cause of death in these patients, use of CXCR1/2 antagonists may potentially lead to enhance severity off bacterial infections if not treated adequately. It has been suggested that CXCR1 is more important for bacterial killing *in vitro* than CXCR2 by human neutrophils, and CXCR1 levels on neutrophils are reduced in CF patients and correlate to decreased antibacterial activity [182]. However, it is not known whether there is differential role for CXCR1 and CXCR2 in dealing with bacterial infections *in vivo*.

Pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) results from abnormal lung healing and excessive collagen deposition on alveolar walls and lung interstitium in response to an injury to alveolar epithelium [187]. The injury can be a result of neutrophil migration into the airways [188]. IPF patients presented increased CXCL8 levels on the airways, which were associated with neutrophilic infiltration and disease severity [188,189]. Bleomycin-induced lung fibrosis is an experimental model to study lung injury and fibrosis [190]. In this model, CXCL1 is produced in early time points following bleomycin instillation and correlates with lung neutrophil accumulation [191], whereas CXCL2 is produced in later time points and correlates with lung collagen deposition [192]. The use of anti-CXCL2 [192] or the CXCR1 and CXCR2 antagonist DF2162 [191] during bleomycin-induced pulmonary fibrosis reduced collagen deposition and angiogenesis. CXCL6 is also involved in IPF and in bleomycin-induced lung fibrosis [193]. High CXCL6 levels are found in bronchoalveolar lavage fluid from IPF patients and in bleomycin-instilled mice; anti-mCXCL6 mAb treatment reduces lung neutrophil infiltration in early stages and lymphocyte recruitment and collagen deposition in later time points [193]. Hence, CXCL6 via CXCR1/2 might mediate neutrophil chemotaxis in early time points after bleomycin, and thus the early inflammatory environment evolves to a profibrotic profile and CXCL2 and CXCL6 release that via CXCR1 and CXCR2, contributing to collagen deposition and lung

fibrosis [191–193]. In conclusion, there is evidence for a role of CXCR2-acting ligands in the context of experimental fibrosis. In such models, blockade or absence of these receptors appears to prevent influx of neutrophils but also angiogenesis, likely by acting on endothelial cells [52,191]. Interestingly, at least one study has shown that blockade of CXCR2 or its ligands has therapeutic effects, that is, protection even when given many days after the inciting stimulus. There is much concern on whether experimental models of fibrosis in rodents really mimic the disease in humans [194], so it is unclear whether experimental findings will translate into benefits for patients. Moreover, as these are very chronic diseases on an already damaged tissue, there are potentially safety concerns on whether blockade of neutrophil influx will facilitate bacterial infection, as for many of the other chronic conditions described here (asthma, COPD, arthritis, etc.). Experience with COPD, described below, should help defining the potential limitations of therapies that limit the function of CXCL8 family of chemokines.

Multiple sclerosis

Neuroinflammation is associated to several diseases and includes excessive microglial activation, impaired cerebral perfusion, blood–brain barrier dysfunction and changing neurotransmission. CXCL8 and its related receptors are normally expressed at low levels in CNS, but increase their expression during neuroinflammation [195]. The main cellular types in CNS that express CXCL8 are microglia, astrocytes, oligodendrocytes and endothelial cells. Furthermore, infiltrated cells into the CNS, as neutrophils and lymphocytes, during pathological conditions are also another important source of CXCL8, contributing to the amplification of inflammation [196].

Multiple sclerosis (MS) is characterized by an inflammatory demyelinating disease [197]. In experimental autoimmune encephalomyelitis (EAE), a murine model of MS, CXCR2 and its ligands is upregulated in the brain and spinal cord of mice. These chemokines can be found colocalized with CD31 staining, typically found in endothelial cells, and in the region rich in T cell influx; the treatment with anti-CXCL1 reduced clinical score of EAE due to, in part, the reduction of granulocyte adhered on cerebral microvasculature in mice [198]. A similar result was observed when the blockade of CXCR2 led to reduced polymorphonuclear leukocyte infiltration into the CNS, reflecting the protection of blood–brain barrier breakdown and less clinical score in EAE [199]. However, CXCR2 is constitutively expressed on oligodendrocytes, the cell type responsible for myelin production, in both mice [200] and humans [201]. Human astrocytes from MS patients produce high amounts of CXCL1, which, in turn, can recruit oligodendrocyte precursor cells to the site of the lesion, suggesting a protective role of this chemokine in MS [201]. However, Lindner and collaborators did not show a functional role of CXCR2 in remyelination process using the cuprizone demyelination model [202], under IL-1β stimulation. The same phenotype was also observed in transgenic mice that overexpress CXCL1 [203]. In another study, CXCL1–CXCR2 interaction avoided oligodendrocyte precursor cells to die by apoptosis when stimulated with IFN-γ [204]. Therefore, although blockade or absence of CXCL8 and its receptors may prevent disease in certain models, these molecules may also be relevant to the remyelination process, making it difficult to develop blockers of this system to treat MS.

Organ transplantation

The process of allograft rejection is related to an intense chemokine and adhesion molecules-dependet recruitment of leukocytes from the innate or adaptive immune system into the allograft [205]. Another important event that contributes to organ dysfunction and morbidity after transplantation is I/R injury in the graft organ that is caused by an excessive inflammatory response, intense neutrophil recruitment, activation and induction of oxidative stress [206]. There are several evidences suggesting a role for CXCL8, CXCR1 and CXCR2 in the context of transplantation. The presence of the polymorphism CXCR1-2668 GA/AA in the CXCR1 gene in the donor is a risk factor for rejection during kidney transplantation [205]. During hepatocyte transplantation, CXCL1, CXCL2, CXCR1, CXCR2 and other chemokines lead to increased neutrophil infiltration and graft rejection [207]. CXCL3, CXCL7 and CXCL8 were found in high levels in transplanted patients with I/R injury, and the role of ELR+ CXC chemokines was demonstrated in a rat orthotopic lung transplantation model that presented high levels of CXCL1 and CXCL2, lung neutrophil sequestration and CXCR2 expression after allograft or isograft lungs after transplantation in response to I/R lung injury. CXCR2 blockade reduced rat postlung transplantation allograft I/R injury [208]. To investigate in which cell type CXCR2 expression was more important to repair, a study with CXCR2 chimeric mice showed that the expression of CXCR2 played a role in liver regeneration in both hepatocytes and myeloid cells, but myeloid CXCR2 was the primary regulator of liver recovery following I/R injury [144]. Five clinical trials using CXCR2 antagonists to improve transplantation outcome are ongoing or completed as described in the clinical trials section and in Table 3. There is reported data for the CXCR2 antagonist reparixin, which restored glycemic levels after islet transplantation in mice and in single infusion islet transplanted patients improved transplant outcome [209]. These studies do suggest that targeting CXCL8 family of cytokines and chemokines is valid in the context of transplantation. Clinical studies should define in detail the diseases or conditions that would gain the most from blocking this chemokine system.

Pain

Pain is an unpleasant but very important sensation to alert the individual from a potential or existing tissue injury [210]. Chemokines, especially the CXCR1 and CXCR2 ligands, have been characterized as important mediators for pain sensation. Injection of CXCL1 in the paw or knee of mice elicits

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nociceptive (index of pain in animals) behavior, which is associated with the release of important mediators that directly stimulate specific primary nerve fibers [211]. Outside the periphery, these mediators also contribute for pain in the CNS. Using murine astrocyte culture, researchers detected that these cells can be a source of CXCL1 under TNF-α stimulation [212]. In a model of neuropathic pain, CXCR2 is upregulated in dorsal horn neurons and the inhibition of TNF-α reduced CXCL1 mRNA synthesis in spinal cord. Moreover, the blockade of CXCR2 decreased CXCL1-induced heat hyperalgesia [212]. However, there is another view about the role of neutrophils and the complex CXCR2 ligands in eliciting pain. Neutrophils recruited by or stimulated with CXCR2 ligands are an important source of endogenous opioids, including met-enkephalin and β-endorphin, especially in early inflammatory response, which in turn have an antinociceptive role [213,214]. Thus, pain triggered by CXCR2 ligands and neutrophil recruitment vary according to the stimulus and time evaluated, demonstrating the complexity of this system in terms of new strategies on development to modulate this sensation. Altogether, the data presented above suggest that the CXCL8 family of chemokines and receptors contributes to pain sensation, which is a major symptom of several diseases. Whether they will add to current available treatment for pain is not known.

Psoriasis

The development of skin inflammatory disorders is thought to be the consequence of the interaction between immune cells and keratinocytes, the main cell type found in the epidermis [215]. Skin lesions in psoriasis are characterized by hyperproliferation of keratinocytes, cellular influx and inflammatory mediators, contributing to itch, epidermal thickness, erythematous plaque formation, increased dermal vascularity and scaly skin [216,217]. The expression of CXCL8 receptors, mainly CXCR2, can be found in suprabasal lesional keratinocytes in psoriasis [218–220]. In an autocrine fashion, CXCL8 can activate these cells to produce and release inflammatory mediators and also contribute to inflammation and migration of neutrophils into the lesion sites [221]. Furthermore, the massive accumulation of neutrophils in the *Stratum corneum* of psoriatic patients was positively stained with CXCL8 antibodies, meaning that neutrophils are also an important source of CXCL8 in this disease [222]. Besides its role in cell recruitment, CXCL8 is associated to angiogenesis on the dermal microvasculature of psoriasis, especially in the chronic phase, providing oxygen and nutrients enhancing cellular recruitment. The proliferation and migration of endothelial cells depend on the production of VEGF and its receptors by several stimuli including CXCL8 released by activated keratinocyte or migrated neutrophils [223,224].

The upregulation of CXCL8 and CXCR2 is induced by several other molecules that are also involved in the pathogenesis of psoriasis including TNF-α, IL-17A, IL-22 and IL-33 [225–227]. In experimental models, injection of IL-33 into the ear induced mast cell-dependent skin lesion in mice. In psoriasiform skin lesion in mice, IL-33 stimulates the recruitment of neutrophils to the site of inflammation due in part to the production of CXCL1 in these mice [228]. More recently, IL-36, a member of the IL-1 cytokine family [229] has been characterized as an important mediator associated to skin lesion in psoriasis [230]. Its production by keratinocytes is positively regulated by TNF-α, IL-17A and IL-22 which, in turn, can stimulate the synthesis of other mediators including CXCL8 [231]. Mice that overexpress IL-36α present spontaneous psoriasiform skin lesion. In this system, the depletion of neutrophils in transgenic mice reversed inflammatory severity, showing, again, a very important participation of neutrophils and their chemotatic mediators in psoriatic skin inflammation [230].

Altogether, these studies provide good evidence that high expression of CXCL8 and its receptors contribute to severity of psoriasis in different contexts. Direct demonstration blocking CXCL8 or its receptors ameliorates human psoriasis is still lacking. However, indirect evidence is provided by studies with IL-10, an anti-inflammatory cytokine that controls the release of inflammatory mediators in different conditions and showed that the administration of this cytokine was effective at reducing inflammatory parameters including dermal thickness, keratinocyte proliferation, T cell infiltration and TNF-α source. IL-10 treatment downregulated CXCL8 and CXCR2 synthesis in keratinocytes present in the stratum spinosum of psoriatic patients [220]. mRNAs are potential candidates to control protein synthesis in a broad range of diseases. More recently, mRNA-31 has been studied as a new biomarker in psoriatic skin [232,233]. Interestingly, its inhibition in keratinocytes reduced the expression of different activators of CXCR2 including CXCL1, CXCL5 and CXCL8 in a TNF-α-dependent manner [233].

Rheumatoid arthritis

Arthritis is the general term given to many rheumatological diseases that affect the joints. Apart from distinct etiology, some features are common among different types of arthritis, including cellular migration to the joint, which have a crucial impact on articular inflammation and pain [234]. Rheumatoid arthritis (RA), the most studied articular disease, is a chronic systemic inflammatory condition associated with genetic and environmental factors, but still without a cause and cure, despite significant recent improvements in treatment [235]. There is a strong association between the presence of CXCL8 and neutrophil recruited in synovial fluid or membrane contributing to the severity of disease in patients with RA [236–239]. Synovial cells are very important sources of CXCL8 and may be directly implicated in neutrophil recruitment to the tissue. In this regard, various mediators can stimulate these cells synthesize CXCL8 including TNF-α [240,241]. Of note, *ex vivo* synovial cells from RA spontaneously produce CXCL8 [242]. In line with this, anti-TNF-α

therapy and steroids are able to reduce the levels of this chemokine and consequently granulocyte recruitment to synovial tissue [240,243,244]. The association of TNF-α and CXCR2 has also been studied in experimental models of arthritis and the blockade of CXCR2 protected mice from the deleterious effect of cellular recruitment to the articular cavity, decreasing joint damage and pain [245–247].

Angiogenesis also participates in the process of articular damage during RA, since it facilitates the infiltration of cells to the synovia [248]. In addition to the role of CXCR2 in new blood vessel formation, VEGF, a very important angiogenic factor, is also released from synovial cells and from infiltrated cells like neutrophils [249,250]. In another study, microparticles which are accumulated in synovial fluids from RA patients could release ELR+ CXC chemokines which, in turn, were able to stimulate endothelial cell migration, suggesting a contribution of these mediators to neovascularization in synovial tissue [251]. The blockade of angiogenesis was investigated experimentally. Interestingly, angiostatic agents like IL-13 inhibit endothelial cells growth via a mechanism partially dependent on decreasing CXCL1 production in arthritic joint in rats [252].

Urological & reproductive diseases

In the context of renal disease, the expression of CXCL8 and CXCR1 was found in human renal diseases and allograft rejection including biopsies from crescentic glomerulonephritis, IgA nephropathy, membranoproliferative glomerulonephritis, lupus nephritis, membranous nephropathy and cancer [253]. Expression of these molecules was found on infiltrating inflammatory cells, predominantly neutrophils, and also in nonleukocyte populations, as arterial smooth muscle cells, endothelial cells of peritubular capillaries [253]. In renal allografts, the expression of CXC occurs mostly during reperfusion, and levels of CXCL8 correlate positively with the ischemic period imposed on the renal graft [254]. The tissue injury may be attenuated by strategies targeting the recruitment of neutrophils. Experimentally, the inhibition of the chemokine receptor CXCR2 by Repertaxin prevents kidney graft deterioration induced by I/R in rats [255]. Moreover, the CXCR2 receptor antagonist Meraxin protects rats from I/R injury in a model of kidney transplantation [256]. In a rat model of acute renal damage, the treatment with dnCXCL8, a human CXCL8-based antagonist designed to generate a dominant-negative mutant protein that enhanced binding to GAGs and reduced CXCR1/2 receptor-binding ability, limited proximal tubular damage and reduced granulocyte infiltration [257]. In rat, acute renal allograft model, dnCXCL8 treatment reduced monocyte and $CD8^+$ T cell infiltration into glomeruli, limiting tubular interstitial inflammation and tubulitis *in vivo*, protecting from chronic kidney dysfunction [257].

CXCL8 may also play a role in other kidney diseases with an inflammatory component including pyelonephritis [258], IgA nephropathy [259] and idiopathic nephrotic syndrome [260]. Data from experimental models also corroborate the hypothesis that the expression of CXCR1/2 and their ligands is deleterious, correlated with kidney injury and inflammation, as demonstrated in a model of nephrotoxic nephritis in rats [261]. Neutralization of rabbit CXCL8 prevented proteinuria, reduced neutrophil influx and glomerular damage in an experimental model of immune complex-induced glomerulonephritis [262]. Using CXCR2 KO mice in DSS-colitis-induced acute kidney injury and inflammation, the expression of cytokines and chemokines and neutrophil infiltration was blunted in the kidney [263]. Polymorphisms in CXCL8 gene is associated with increased risk of nephritis as in children with Henoch–Schönlein purpura [264] and severe systemic lupus erythematosus nephritis [265]. Furthermore, CXCL8 levels have been used as a marker of urinary disease progression, detected in the urine of patients with pyelonephritis [258], IgA nephropathy [259] and idiopathic nephrotic syndrome [260] and used as biomarker for the detection of bladder cancer [266].

There have been several studies showing the relevance of CXCL8 in the context of prostate hyperplasia and cancer [267]. CXCL8 levels are elevated in patients with benign prostatic hyperplasia (BPH) and may be useful as a biomarker of inflammation in BPH [268]. CXCL8 induces autocrine and paracrine proliferation of BPH cells, indicating also a growth-promoting activity of this chemokine in disease pathogenesis [269]. Interestingly, senescence of prostatic epithelial cells was associated with increased expression of CXCL8, which could promote proliferation of nonsenescent epithelial and stromal cells, and contributed to the increased tissue growth seen in BPH [270]. Levels of CXCL8 were significantly increased in seminal plasma from patients with BHP and chronic prostatitis/chronic pelvic pain syndrome, suggesting CXCL8 as predictive marker to diagnose prostate inflammatory conditions such as BPH and chronic prostatitis/chronic pelvic pain syndrome [271]. Elevated levels of CXCL8 have also been found in seminal plasma from males with leukospermia and were related to infertility [272]. High levels of CXCL8 were found in ovarian endometrioma [273] and in peritoneal fluid from female patients with endometriosis [274], suggesting that CXCL8 induced proliferation, growth and survival of endometrial stromal cells [275]. Altogether, these studies clearly show that the CXCL8–CXCR1/2 axis is relevant and may be of potential therapeutic usefulness in the context of urological diseases.

CXCL8 family & receptors: ongoing clinical trials & future perspectives

Available drugs & clinical trials

Currently, 38 clinical trials involving CXCL8, CXCR1 and CXCR2 are registered on clinical trials databases. These trials are ongoing or have been finished and have been conducted to evaluate the potential of these antagonists to treat different diseases (Table 3). Full reports are missing for many of these trials. The first clinical trial that achieved therapeutic benefit was the

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treatment of patients with pancreatic islets transplantation with the CXCR1 and CXCR2 antagonist, reparixin [209], a small molecule noncompetitive allosteric inhibitor [276]. Diabetic CXCR2 KO mice or mice treated with reparixin restored glycemic levels after islet transplantation, reduced hepatic infiltration of neutrophils and natural killer T cells. A Phase II clinical trial was performed to assess the efficacy and safety of reparixin after single infusion islet transplantation. While all the control group patients have withdrawn the trial after 1 month due to lack of β cells function and graft loss, reparixin treated improved transplant outcome and did not cause significant adverse effects [209]. reparixin has been tested in other clinical trials involving organ transplantation and also breast cancer (Table 3).

Based on a previous study in LPS or lavage-induced airway inflammation in mice, rats and cynomolgus monkeys showed that treatment with the CXCR1 and CXCR2 antagonist SCH527123 reduced pulmonary neutrophilia and airway mucin content [277], and the antagonists SCH527123 was tested in healthy volunteers ozone-induced neutrophilia [163] and reduced neutrophils numbers in sputum and in blood and was considered safe and well tolerated in the evaluated doses. In a clinical trial with asthmatic patients, SCH527123 reduced neutrophil numbers, caused milder exacerbations and was considered safe [173]. Although SCH527123 is considered an allosteric CXCR1 and CXCR2 antagonist, the compound is selective for CXCR2 and requires high concentrations to inhibit polymorphonuclear leukocyte chemotaxis through CXCR1 [278].

Another CXCR2 antagonist, SB656933, was also tested in the ozone-induced neutrophilia model in healthy volunteers and presented similar results as SCH527123 [162,163]. Peripheral blood neutrophils isolated from SB656933-treated subjects showed reduced CXCL1-induced CD11b expression, a marker of neutrophil activation [162]. SB656933 was also tested in CF patients for 28 days and was proven to be safe regarding side effects and reduced disease exacerbation, neutropenia and composition of bacteria in sputum. In addition, high doses of the compound reduced neutrophil numbers and activation [185]. A different CXCR2 antagonist, AZD 8309, was used in healthy volunteers subjected to LPS nasal challenge and reduced neutrophil counts in nasal lavage fluid from 99 to 48% and also reduced LTB4 levels and elastase activity, without causing side effects [164].

Three drugs that target the chemokine CXCL8 have been tested in clinical trials: ABX-IL8, HuMab 10F8 and PA401. ABX-IL8 is a fully human anti-CXCL8 antibody from Abgenix that was first tested in a murine melanoma model and inhibited tumor growth and angiogenesis [279]. The pharmacokinetic properties of the antibody have been tested in patients with psoriasis and RA [280], and a Phase II clinical trial with COPD patients was completed but the results were not published in full format, to the best of our knowledge. HuMab 10F8 is a human mAb against CXCL8, which binds a discontinuous epitope overlapping the receptor binding site, neutralizing IL-8-dependent effects. HuMab 10F8 was beneficial to patients suffering from palmoplantar pustulosis, a chronic inflammatory skin disease. In these patients, HuMab 10F8 was well tolerated and significantly reduced clinical disease activity, with a >50% reduction in the formation of hand pustules. HuMab 10F8 represents a candidate for treatment of inflammatory diseases and other pathological conditions associated with CXCL8 overproduction [281]. The company, ProtAffin, developed a modified form of CXCL8 called PA401 with increased binding affinity to glycosaminoglycans and decreased ability to bind and activate CXCR1/2 and was tested *in vivo* in models of acute renal allograft damage and lung inflammation with positive results [282]. The Phase I clinical trial aimed to evaluate safety and tolerability of PA401 in healthy volunteers, and the results have not been published yet.

Expert commentary

CXCL8 family of chemokines and receptors has been traditionally associated with neutrophil activation and recruitment into the tissue and neutrophil-dependent tissue damage. Therefore, much of the effort in the development in the area has focused on diseases with a clear neutrophil influx and association with presence of CXCL8. However, it is clear that CXCR1 and CXCR2, the receptors for CXCL8 and related chemokines, are present on other cell types – including endothelial, epithelial, glial and parenchymal cells and other leukocytes – and may have a role in disease progression. Hence, the benefits of blockade of CXCR1/2 may extend well beyond controlling neutrophil migration. Inhibition of angiogenesis, tumorigenesis and fibrogenesis are effects seen after blockade of CXCR1 and CXCR2 on cells other than neutrophils and may be beneficial in diverse diseases.

Because of this wide distribution, therapeutic manipulation of the system is clearly beneficial in certain experimental situations (such as transplantation, ALI, COPD, emphysema, chronic bronchitis, bronchiectasis, asthma and CF), but may be detrimental in certain conditions in which CXCR1/2 receptor stimulation has a protective effect such as sepsis, pulmonary infection and MS (Figure 1). This has led to the development of several receptor antagonists, modified chemokines and antibodies that have been tested in several conditions, especially in diseases of the pulmonary system and organ transplantation (Table 3). Most indications tested so far have focused on neutrophilic diseases. As these drugs advance into patients, other clinical indications that bear in mind the wider distribution of CXCR1/2 may emerge. For example, the potential benefit of blocking the CXCL8 family and receptors in cancer extend well beyond the expression of CXCR1/2 on neutrophils. The expression of these receptors on cancer cells and endothelial cells is likely to be more important for any therapeutic benefit. Whether such non-neutrophilic role of the CXCL8 family may be exploited for therapeutic in other chronic inflammatory diseases, such as arthritis and fibrotic diseases, clearly deserves further investigation.

At present, it is difficult to differentiate the relevance of CXCR1 and CXCR2 for the actions of the CXCL8 family of

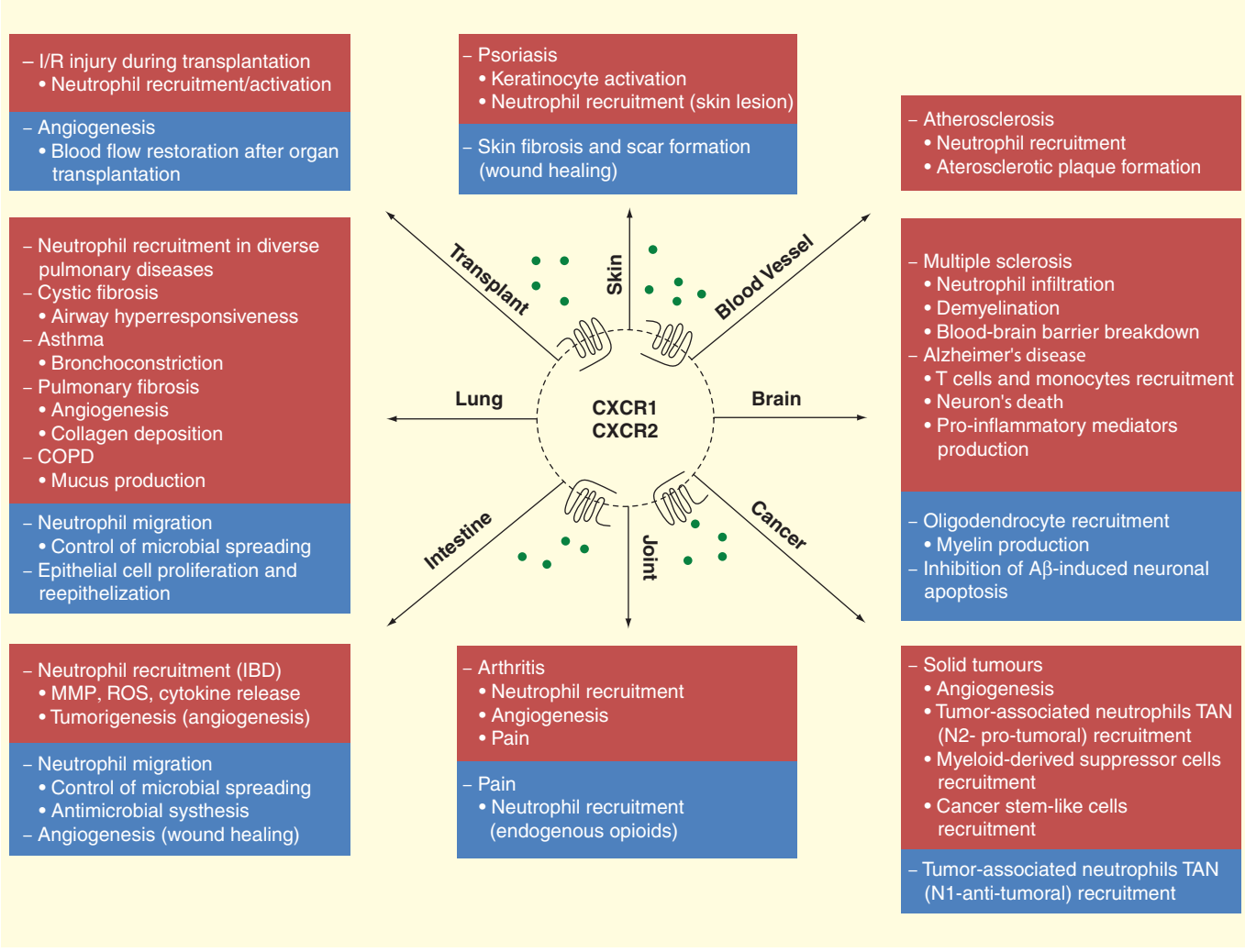

Figure 1. Central role of CXCR1 and CXCR2 mediating organ-specific pathologies. CXCL8 family and CXCR1/CXCR2 receptors regulate various specific organs diseases, most deleterious effects are associated with high intense and chronic immune response and tissue damage (red boxes), also by activation of parenchyma cells. However, there are some positive effects in terms of activation of CXCR1/CXCR2 receptors, leading to tissue homeostasis adequacy (blue boxes), counterbalancing tissue injury in diverse pathologies by a pleiotropic role of CXCL8 family.
COPD: Chronic obstructive pulmonary disease; IBD: Inflammatory bowel diseases; MMP: Metalloproteinase; ROS: Reactive oxygen species.

chemokines *in vivo*. This is compounded by the fact that there are few experiments *in vivo* evaluating the role of rodent CXCR1. Therefore, the precise role of the latter receptor and whether differential or combined target of CXCR1 and CXCR2 would be advantageous for treating inflammatory diseases is unknown. Similarly, it is not known whether blocking CXCL8 activity alone (by using anti-IL-8 antibodies) would be as effective as blocking the receptors and with fewer infections side effects. There is much redundancy of the binding of members of the CXCL8 family on CXCR2, but not CXCR1. It is not known at present whether there is a therapeutic opportunity to be exploited from the differential action of these chemokines on these receptors.

Finally, one must keep in mind that CXCR1/2 is necessary for neutrophil influx in various models of bacterial and fungal infection and spread (Figure 2). Therefore, one potential side effect from administration of drugs that modifies the CXCL8 chemokine family and receptors is the occurrence of bacterial and fungal infections. This should not deter the use of these drugs, but trials should have this potential in mind when examining for potential side effects, especially when treatment is prolonged.

Five-year view

In the years to come, advances of genetic engineering associated to animal disease modeling will deepen our knowledge of the biological functions of CXCL8 family members and receptors. For example, mice deficient/reporter for CXCL8 family members in specific tissues or cell types may help identifying particular cell types producing and responding to these molecules. This is particularly relevant to identify neutrophil-dependent

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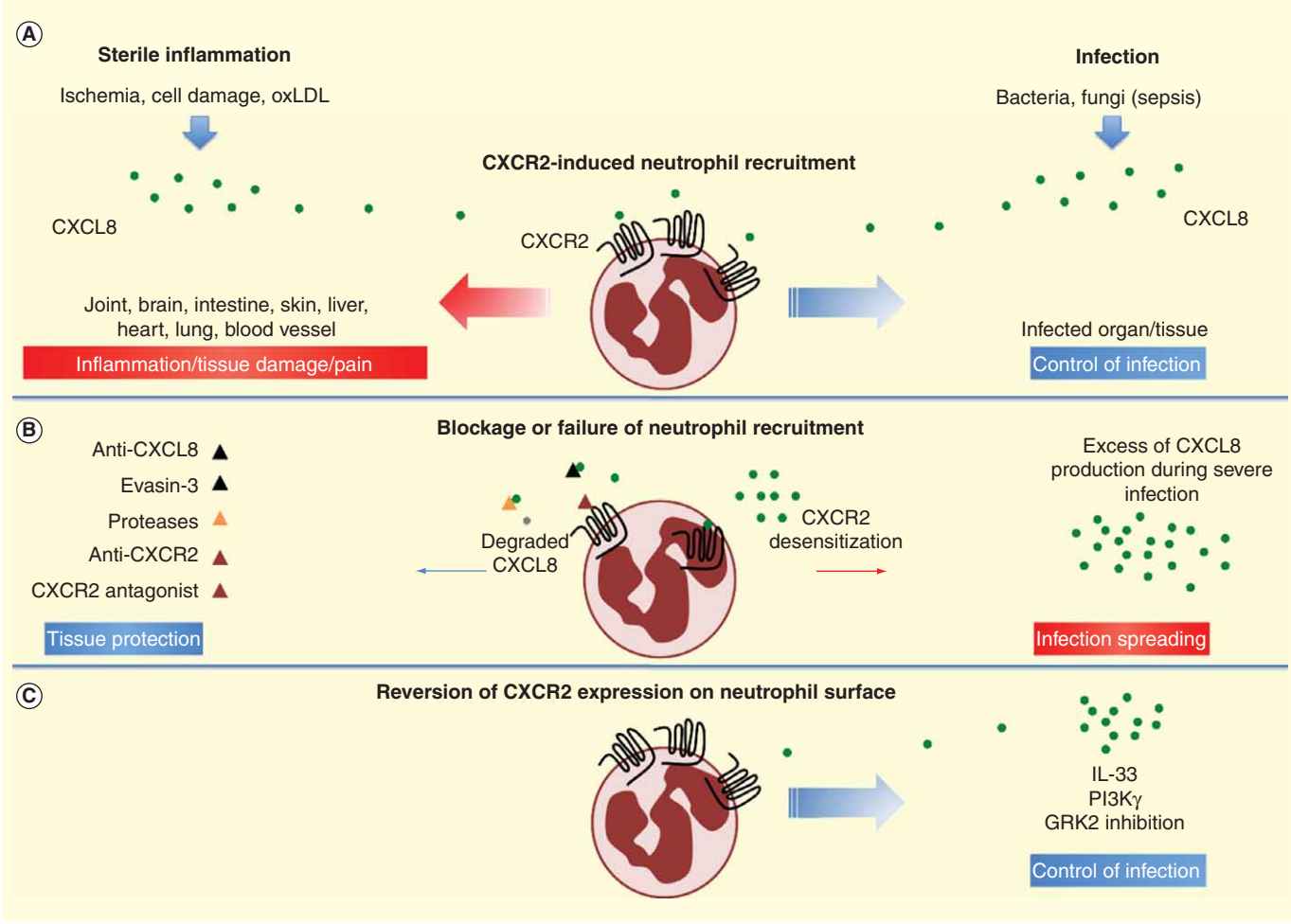

Figure 2. CXCR2-induced neutrophil recruitment in different context of inflammation. (A) Several stimuli can elicit CXCR2-binding ligands production by local cells and promote neutrophil recruitment to the tissue. Although migrated neutrophils are very important to deal with pathogens during infection, the inflammatory mediators released by these cells contribute to tissue damage, also in noninfection diseases, amplifying inflammation and causing pain. **(B)** Impairment of neutrophil recruitment can be used as a therapeutic strategy to avoid tissue damage and dysfunction in noninfectious diseases. In severe infections, high production of CXCR2 ligands increases desensitization of its receptor on cell surface after chemokine: receptor interaction, impairing neutrophil migration promoting infection spreading. **(C)** Future strategies to prevent CXCR2 desensitization by target-specific molecules responsible for this event (IL-33, PI3Kγ, GRK2) can be an alternative option to avoid decreased neutrophil to the site of infection under excessive CXCR2-binding chemokines. OxLDL: Oxidized low-density lipoprotein.

and independent actions of CXCL8 family members and to pinpoint actions of these molecules on cells other than leukocytes. As several clinical trials are completed, the potential usefulness of blocking CXCL8 for the treatment of certain chronic inflammatory and autoimmune diseases will be available. These findings and the availability of safe drugs/antibodies may open opportunities for targeting CXCL8 in previously unanticipated diseases.

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Key issues

- CXCL8 was first cloned as a neutrophil-specific chemoattractant.
- In addition to CXCL8, the CXCL8 family comprises the chemokines CXCL1, CXCL2, CXCL3, CXCL5, CXCL6 and CXCL7, which share a common ELR (glutamic acid–leucine–arginine) motif and the CXCR1 and CXCR2 receptors.
- CXCR1 and CXCR2 are expressed on neutrophils at high levels but also on other leukocytes and other cell types. Hence, the benefits of blockade of CXCR1/2 may extend well beyond controlling neutrophil migration.
- There is much data demonstrating the expression and role for the CXCL8 family of chemokines in acute and chronic inflammatory conditions and cancer. These molecules may be, however, relevant for host immune responses against certain infections.
- Several inhibitors or antagonists for the CXCL8 family are available and currently being tested for the treatment of several acute and chronic inflammatory conditions of humans.

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- **Interesting paper demonstrating that a particular miRNA (miR31) can regulate the expression of CXCR1/2 ligands such as CXCL1, CXCL5 and CXCL8/IL-8 in human keratinocytes.**

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